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14. ABSTRACT

The TGF-α transgenic over expression model has been used to study circadian running wheel activity (RWA) in C57Bl6 mice. The genotype of each transgenic animal is determined by PCR analysis of tissue and gel electorphoresis. Male and female transgenic and control animals have been assessed for RWA, phase, and total amount of activity in a light controlled environment using a 12:12 hour light/dark (LD) cycle and in constant darkness (DD). The results show an intact central clock in LD and DD condition in each gender when assessed for phase of the circadian period. The endogenous rhythm of both the transgenic and wild type animals is 23.45 and 23.55 hours for female and males animals, respectively. The measure of the relative duration of activity in the transgenic animals is significantly shorter compared to controls and is consistent with our hypothesis that overproduction of TGF-α is associated with inhibition of activity. Our next phase of study will use this established model to test the effect of a tyrosine kinase inhibitor on activity.

15. SUBJECT TERMS
Transgenic, TGF-2, EGFR1, Symptom research

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Introduction:

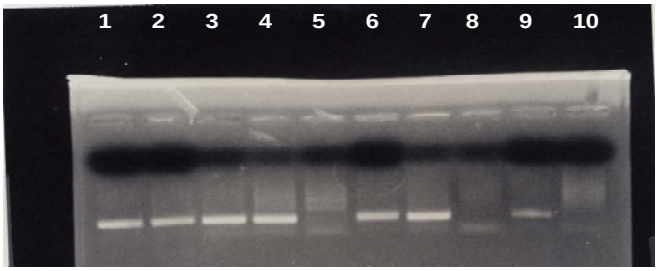
Symptom clusters in cancer patients include fatigue, appetite loss, and sleep disruption. Fatigue is the most common symptom and the cause is likely related to multiple factors. One mechanism for fatigue that has been recently elucidated is the loss of rhythmic circadian activity in animals that receive an intracerebral-ventricular infusion of ligands of the epidermal growth factor receptor, EGFR [1,2.3]. This receptor has been shown to control several rhythmic behaviors in the laboratory rodent. We hypothesize that animals that over express the ligand of the EGFR would show measurable signs of decreased activity.

Body:

Our laboratory has commenced studies on circadian running wheel behavior of transgenic mice overexpressing TGF-α. This work began with setting up a breeding colony of transgenic mice in our core vivarium facilities. The mice started producing offspring after the age of 6 weeks and because of the propensity for these mice to develop tumors after the age of 6 months we have not kept breeding pairs active beyond this age. However, after starting up our breeding colony and having success we noticed that mice after the age of approximately 4-5 months become less fertile and we will retire the breeding pairs after the age of 4 months from continued breeding.

Once the offspring are weaned they are ear tagged and assessed for genotype. The genotype procedure consists of snipping a small piece of the distal tail under auspices of an approved ACUC animal protocol. The tail piece is digested and assessed for the TGF-α gene expression using a PCR technique. In the figure below are shown examples of transgenic TGF-α overexpression mice where the bright band in the electrophoresis gel demonstrates the presence of the gene in each animal. Once each animal has been genotyped it is now ready to be used in the running wheel experiments.

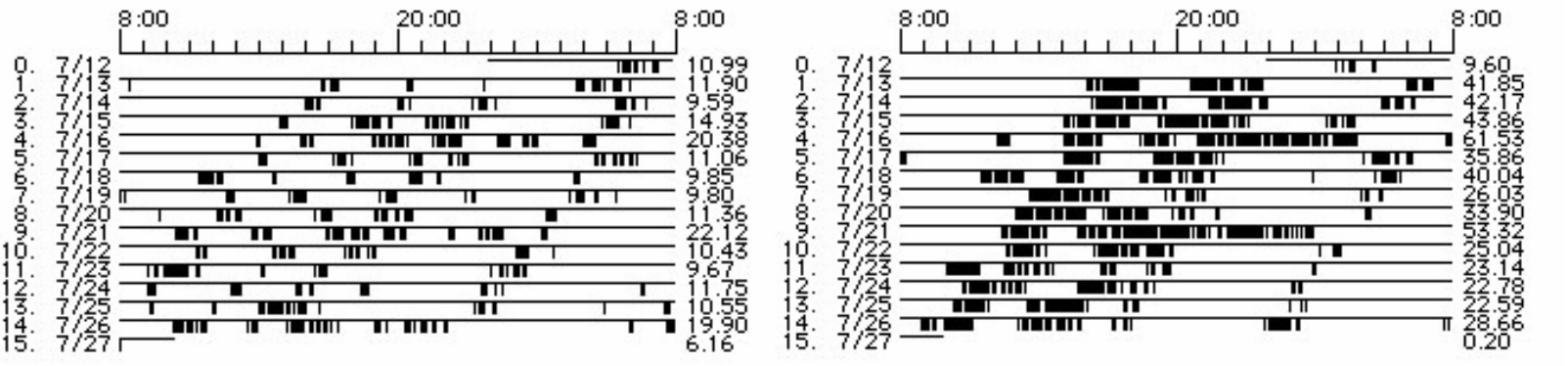
Transgenic TGF-α overexpression model:



PCR Analysis:
Lanes 1-4, 6-7, and 9 all show positive results indicating TGF-alpha expression.
Lanes 5, 8, and 10 show a negative result and can be used as control mice.

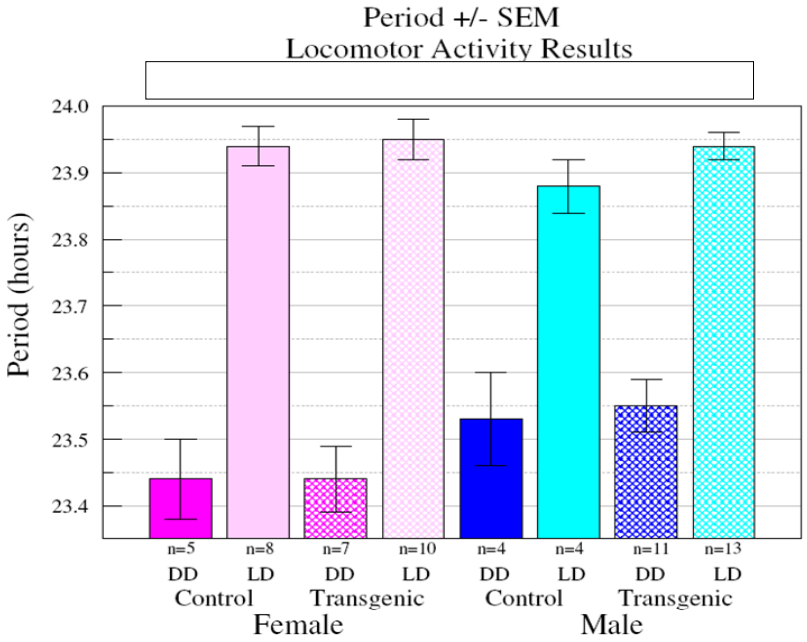
Running wheel experiments are performed in a specialized light and temperature controlled environment and where the daily activity is recorded on a specialized computer log. One delay in initiating our project was the institution of a new electronics system which required several months to debug. Once we discovered that there was an incompatibility between the motherboard and the PC board in the first computer, we have not experienced any further problems. This, however, did result in loss of time in being able to obtain usable data.

Shown below is an actigram of a transgenic TGF-α mouse in a constant darkness.

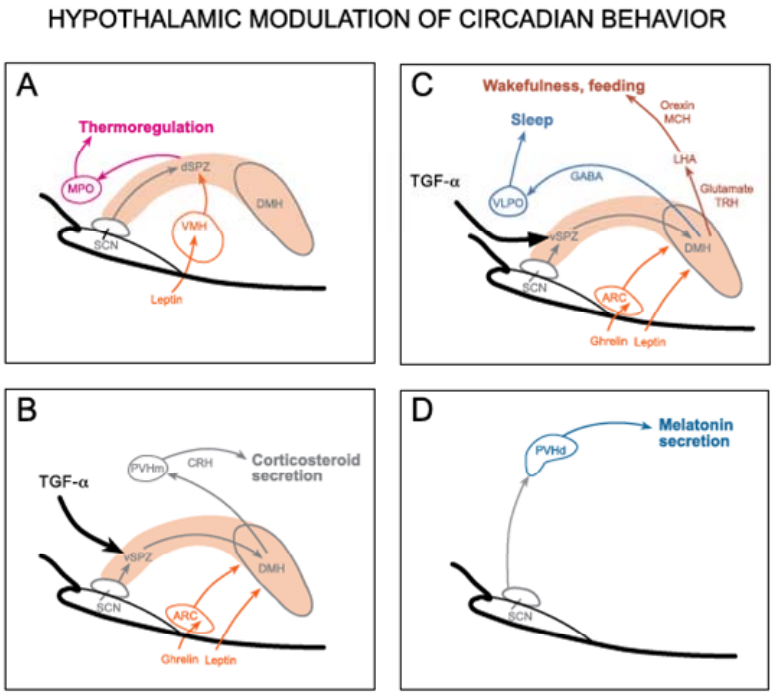


animal and the control is on the right. These figures show less running wheel activity in transgenic compared to the wild type.

The next two figures are an example of statistical analysis with sufficient numbers of mice per group (LL and LD stand for 12 hours lights on and 12 hours darkness; the DD stands for constant darkness) showing that the running wheel behavior for the experimental TGF-α overexpression mice compared to the controls shows no statistical difference between the period of the running wheel behavior in either LL or LD conditions.

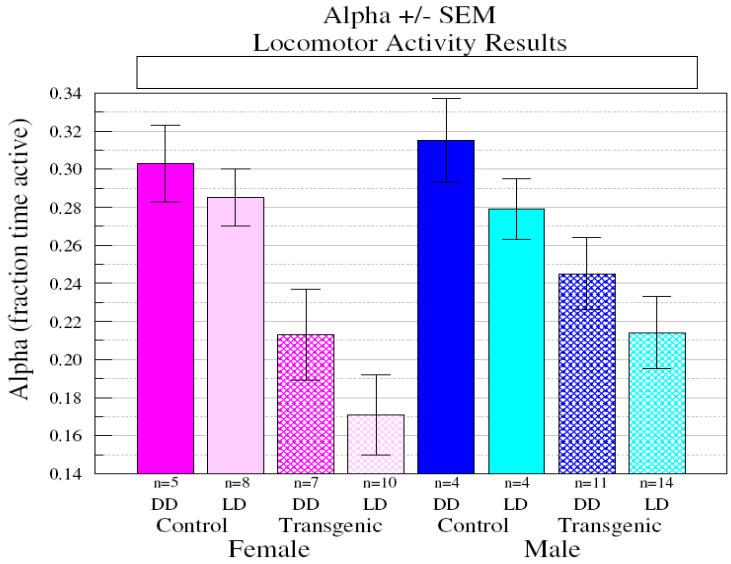


Interpretation of this figure is consistent with the notion that running wheel behavior generated by the activity from the suprachiasmatic nucleus is no different in the transgenic overexpression TGF-α animals compared to the controls. This is consistent with our hypothesis shown in the next figure where both rat and hamster hypothalamic nuclei ablation data suggests that the modulation of the circadian running wheel behavior occurs downstream from the level of the central clock in the SCN [4].



The model shown here is consistent with modulation of the clock signal downstream from the SCN at the vSPZ nucleus in the hypothalamic paraventricular region.

The fourth panel shows evidence for a decrease in activity in the transgenic animals regardless of lighting conditions. This suppression of activity is consistent with our hypothesis shown in the last figure.



Conclusion: Controls exhibit more relative active time than do transgenics.

Alpha is a measure of the relative duration active (a value of zero meaning no activity, a value of unity corresponding to no rest).

p-value $_{2\text{-sided}} < 0.0001$ (Welch's t-test used because variances not equivalent; p(SD) = 0.0011)

Based on this model, we are moving forward with studies where we will inject a EGFR tyrosine kinase inhibitor and measure any changes in activity. We have obtained a commercial tyrosine kinase inhibitor, AG1478, that is a lipophilic tyrosine kinase inhibitor of the EGFR family. Other published studies indicate that this has excellent brain penetration. We will also conduct experiments with another tyrosine kinase inhibitor, Tarceva because of the clinical relevance of such studies.

Key research accomplishments:

In spite of having difficulties with the transgenic mouse breeding colony and production of sufficient numbers of transgenic mice and with electronic difficulties in the initial setup of the computerized running wheel system, we have obtained statistically significant data that supports our hypothesis. The data show that the TGF-α transgenic animal has a suppression of overall activity which is consistent with the "lesion" at the hypothalamic nucleus downstream from the SCN where the presence and excess TGF-α produces decreased running wheel activity.

Reportable outcomes: These initial results are consistent with our hypothesis. They will be included in a larger manuscript cover the EGFR transgenic animals and to also include the results of intervention with EGFR inhibitors.

Conclusion:

The data we have obtained support our hypothesis that the peripheral production of TGF-α in the transgenic over cxpression model are associated with loss of activity. This is predicted by the model based on neuroanatomic studies and functional activity of ligands of the EGFR in the circadian signaling pathway. The relevance to cancer treatment is the potential use of tyrosine kinase inhibitors in the targeted management of symptoms in patients.

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